This report is required by law (7 USC 2143). Failure to report according to the regulations can result in an order to cease and desist and to be subject to penalties as provided for in Section 2150.

See reverse side for additional information.

Interagency Report Control No 0180-DOA-AN

UNITED STATES DEPARTMENT OF AGRICULTURE
ANIMAL AND PLANT HEALTH INSPECTION SERVICE

ANNUAL REPORT OF RESEARCH FACILITY
(TYPE OR PRINT)

1. REGISTRATION NO. 93-V-0004 CUSTOMER NO. 1323

FORM APPROVED OMB NO. 0579-0036

2. HEADQUARTERS RESEARCH FACILITY *(Name and Address, as registered with USDA, Include Zip Code)*

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3. REPORTING FACILITY (List all locations where animals were housed or used in actual research, testing, teaching, or experimentation, or held for these purposes. Attach additional sheets if necessary.)

FACILITY LOCATIONS*(sites)*

Veterinary Medical Unit (VMU)

REPORT OF ANIMALS USED BY OR UNDER CONTROL OF RESEARCH FACILITY *(Attach additional sheets if necessary or use APHIS FORM 7023A)*

A. Animals Covered By The Animal Welfare Regulations	B. Number of animals being bred, conditioned, or held for use in teaching, testing, experiments, research, or surgery but not yet used for such purposes.	C. Number of animals upon which teaching, research, experiments, or tests were conducted involving no pain, distress, or use of pain-relieving drugs.	D. Number of animals upon which experiments, teaching, research, surgery, or tests were conducted involving accompanying pain or distress to the animals and for which appropriate anesthetic, analgesic, or tranquilizing drugs were used.	E. Number of animals upon which teaching, experiments, research, surgery or tests were conducted involving accompanying pain or distress to the animals and for which the use of appropriate anesthetic,analgesic, or tranquilizing drugs would have adversely affected the procedures, results, or interpretation of the teaching, research, experiments, surgery, or tests. *(An explanation of the procedures producing pain or distress in these animals and the reasons such drugs were not used must be attached to this report)*	F. TOTAL NO. OF ANIMALS (Cols. C + D + E)
4. Dogs					
5. Cats					
6. Guinea Pigs					
7. Hamsters					
8. Rabbits			717	9	726
9. Non-Human Primates					
10. Sheep					
11. Pigs					
12. Other Farm Animals					
13. Other Animals					

ASSURANCE STATEMENTS

1) Professionally acceptable standards governing the care, treatment, and use of animals, including appropriate use of anesthetic, analgesic, and tranquilizing drugs, prior to, during, and following actual research, teaching, testing, surgery, or experimentation were followed by this research facility.

2) Each principal investigator has considered alternatives to painful procedures.

3) This facility is adhering to the standards and regulations under the Act, and it has required that exceptions to the standards and regulations be specified and explained by the principal investigator and approved by the Institutional Animal Care and Use Committee (IACUC). A summary of all the exceptions is attached to this annual report, in addition to identifying the IACUC-approved exceptions, this summary includes a brief explanation of the exceptions, as well as the species and number of animals affected.

4) The attending veterinarian for this research facility has appropriate authority to ensure the provision of adequate veterinary care and to oversee the adequacy of other aspects of animal care and use.

CERTIFICATION BY HEADQUARTERS RESEARCH FACILITY OFFICIAL
(Chief Executive Officer or Legally Responsible Institutional official)
I certify that the above is true, correct, and complete (7 U.S.C. Section 2143)

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DATE SIGNED 11/25/08

EADQUARTERS

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One VASDHS IACUC approved protocol involves a category E study in rabbits. This study is conducted in accordance with USDA, NIH and AAALAC, International guidelines and policies for the humane care and use of research animals. The protocol involves a well documented stroke model (cerebral ischemia) in rabbits. Stroke is produced by injecting blood clots into the carotid artery causing cerebral ischemia. The procedure used to gain access to these vessels are performed under general anesthesia using aseptic technique and would be considered USDA category D. Animals are allowed to fully recover from anesthesia prior to inducing the ischemic condition; animals are restrained in a standard rabbit restrainer for the injection. Although stroke is reportedly not painful in humans, it has the potential of being distressful, if severe. It is for this reason that these studies are identified as USDA category E studies. This model was previously used to develop the thrombolytic, tissue plasminogen activator, the only FDA-approved treatment for stroke. To develop treatments, it is necessary to use a model system that accurately reproduces human stroke and has a reproducible clinical endpoint to monitor.

Animals are allowed to recover from anesthetic prior to producing stroke because of anesthetic and hypothermic effects and to have a more relevant model by producing stroke in an awake animal. Anesthetics that interact or regulate potassium channels in the CNS play an important role in neuroprotection against brain and spinal cord ischemia (Heurteaux et al., EMBO J. 2004 Epub). The literature indicates that reducing body temperature by 1 or more degrees may be neuroprotective (Smith J. Vasc. Interv. Radiol. 15:S3-12, 2004). Since many anesthetics reduce body temperature, it is likely that neuroprotection would occur and interfere with the model. Further, novel targets for therapeutic intervention include compounds that reduce the inflammatory cascade and apoptotic mechanisms, including non-steroidal anti-inflammatory drugs, cyclo-oxygenase inhibitors (COX-2) and platelet-adhesion antagonists. We are also focused on early inhibition of the ischemic cascade and are studying compounds that reduce excitotoxicity and inhibit the deleterious effects of free radicals. Typically, two classes of compound are used as pain-relievers in experimental animals. They include morphine analogs and NSAID's. Neither class of compound can be used in our experimental ischemia studies for many reasons, including their ability to confer neuroprotection via a variety of mechanisms including reducing inflammation. Since we are actively developing novel thrombolytics for the treatment of acute ischemic stroke compounds that affect blood chemistry especially the clotting mechanisms (e.g. aspirin), will interfere with the assessment of thrombolytics. A brief review of the scientific literature shows that simple compounds such as aspirin confer neuroprotection in models of CNS disease (Teismann and Ferger. Synapse 39: 167-174, 2001; Grilli et al., Science 274: 1383-1385; Vartiainen et al., J. Neurochem. 82: 329-335, 2002). Moreover, other aspirin-like platelet inhibitors can reduce ischemic brain damage (Moriguchi et al., JPET 308: 1094-1101, 2004). Historically, morphine and its analogs including buprenorphine have been known to be neuroprotective (Mastronardi and Cafiero, Minerva Anesthesiol. 67:332-7, 2001). The authors point out that that delta-opiate receptor compounds confer a preconditioning-like protective effects against myocardial ischemia mu-opiate and kappa-opiate receptors are involved in ischemic preconditioning against seizures in the brain. Moreover, a recent publication showed that treatment of rats with buprenorphine reduced lesion-induced cell death (Ozden et al., J. Neurotrauma 21: 73-82, 2004).Taken together, the scientific literature clearly shows that systemic intervention with analgesics and/or anesthetics in our models may obscure and interfere with the studies of new therapeutics to treat cerebral stroke.

Following induction of an ischemic stroke, animals are kept in the lab under close observation for at least 2 hours. A post-procedure behavioral scale (scaling 0-5) is used when monitoring the animals to rate the severity of the stroke. If it is determined that the severity of stroke is such that there is a potential that animals may not be able to eat or drink or if they may be in pain or distress, per the objective scoring behavioral scale, they are euthanized. Animals are monitored and evaluated daily by veterinary and laboratory staff utilizing the post-procedure behavioral scale and ensuring animals are able to eat and drink. Animals assigned to category E are those that rate at the high (3-5) end of the scale but are able to eat, drink and ambulate in the cage. A typical study requires the use of 20-24 rabbits based upon power analysis calculation to complete a quantal analysis curve. Therefore, a complete study, including 3-4 treatment groups, required approximately 80 rabbits. Historically, approximately 20% are euthanized within the first two hours due to severity of the stroke (3-5 on our scoring scale). However, due to extensive honing of the model, we have found that out of every 80-100 rabbits approximately 4-5 rabbits must be euthanized after the first two hours or before the completion of the study due to severity of stroke and/or potential distress. During this reporting cycle animals 9 were humanely euthanized due to severity of stroke and/or potential distress and are reported as category E.

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The following outlines an IACUC approved deviation from USDA regulation. During this reporting cycle 186 rabbits were pair housed.

VASDHS IACUC POLICY
Pair Housing Rabbits; Deviation from USDA Floor Space Requirements

According to the 1996 *Guide for the Care and Use of Laboratory Animals*: (page 22)

> "Animals should be housed with a goal of maximizing species-specific behaviors and minimizing stress-induced behaviors. For social species, this normally requires housing in compatible pairs or groups. A strategy for achieving desired housing should be developed by animal-care personnel with review and approval by the IACUC. Decisions by the IACUC, in consultation with the investigator and veterinarian, should be aimed at achieving high standards for professional and husbandry practices considered appropriate for the health and well being of the species and consistent with the research objectives. After the decision- making process, objective assessments should be made to substantiate the adequacy of animal environment, husbandry, and management."

In order to provide pair housing to rabbits using the VMU's existing caging, deviation from the minimum space requirements in the Guide and the Animal Welfare Regulations is necessary. Both documents recommend 3 sq. ft. for rabbits up to 4 kg, however, the VMU's cages provide 5 sq. ft. of floor space.

It is believed that pair housing young rabbits reduces the stress of the new cage environment and the incidence of mucoid diarrhea associated with stress.

Pair housed young rabbits in existing 5 sq. ft. caging can be seen grooming one another, resting side by side, and sharing feed at the feeder demonstrating that the animals are content in the space provided.

The following procedures are followed for pair housing rabbits:

1. Pairs will be housed in 6 sq. ft. caging when available.

2. Rabbits received at 2.75 kg or less will be housed in compatible same-sex pairs. When paired rabbits become more than 3 kg in weight, they will be singly housed.

3. Rabbits that have undergone a surgical procedure will be housed singly until sutures are removed or for the remainder of the study, according to the needs of the study.

4. Investigators will be responsible for ensuring that pair housed animals are permanently identified if necessary for their study.

All animals are observed daily by VMU Animal Health Staff. Paired rabbits that begin to fight or aggressively compete for food will be separated and housed individually.

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